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7-OXO-, 7α-HYDROXY- AND 7β-HYDROXYSTEROLS FROM *EUPHORBIA FISCHERIANA*

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Key Word Index *Euphorbia fischeriana*; Euphorbiaceae; antitumoral drugs; 7-oxosterols; 7α-hydroxysterols; 7β-hydroxysterols.

Abstract—7-Oxo, 7α-hydroxy- and 7β-hydroxysterols (campesterol, stigmasterol and sitosterol derivatives) were isolated from the roots of *Euphorbia fischeriana*, a drug used for its antitumour properties in traditional Chinese medicine. Some of these steroids show antitumour activity and might be related to the presumed activity of the drug.

INTRODUCTION

Euphorbia fischeriana Steud. (= *Euphorbia pallasii* Turcz, Euphorbiaceae [1, 2] is a perennial herbaceous plant, widespread in Mongolia and Siberia, which has been used in traditional Chinese medicine for more than two thousand years as an antitumour drug [3]. At this time, only *O*-acetyl-*N*-(*N*-benzoyl-L-phenylalanyl)-L-phenylalantol has been reported from this plant [4]. We have now analysed extracts prepared from the roots in an attempt to isolate the agents responsible for the pharmacological activity.

RESULTS AND DISCUSSION

The HTC (Hepatoma Tumor Cell) test was used for the determination of the cytotoxic or antitumour activity of fractionated extracts [5]. Only the most polar fractions of the petrol and ether extracts prepared from the dried roots showed slight cytotoxic activity. Fractionation of these extracts by column chromatography and preparative TLC led to the isolation of three fractions, each corresponding to a mixture of the 7-oxo- (**1b, 2b, 3b**), 7α-hydroxy- (**1c, 2c, 3c**) and 7β-hydroxy- (**1d, 2d, 3d**) derivatives of campesterol (**1a**), stigmasterol (**2a**) and sitosterol (**3a**), which were also found as the free sterols. All steroids were identified by their GC retention times, GC–MS of their TMSi-ether derivatives and their 250 MHz ^{1}H NMR spectra which were identical [5, 6] to those of the corresponding compounds synthesized from stigmasterol and a commercial mixture of campesterol and sitosterol.

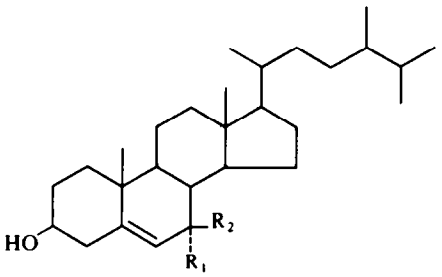
Autoxidation of Δ^5-sterols occurs preferentially at the allylic C-7 position [7, 8]. The composition of all steroid fractions isolated from *E. fischeriana* are identical (Table 1). It seems, therefore, that the three fractions of steroids oxygenated at C-7 are derived from a non-selective autoxidation of the parent compound or from a non-selective biological oxidation. 7-Oxo- and 7-hydroxysterols have only rarely been looked for and identified in plants. For instance, 7-oxositostérol and 7-oxostigmasterol were found in *Phaseolus vulgaris* [9]. Their presence in *E. fischeriana* is therefore interesting and these steroids may represent a class of compounds more widespread in plants than is generally believed.

Table 1. Relative amounts of phytosterol derivatives in each steroid fraction isolated from *E. fischeriana*

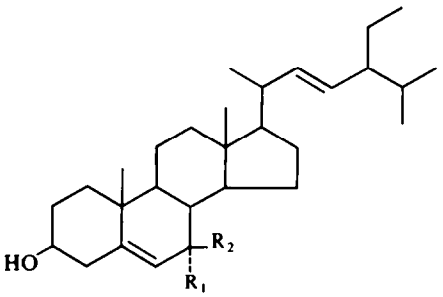
Steroid fractions	Derivative of camp-esterol (%)	stigm-asterol (%)	sito-sterol (%)
Esterified sterols	23	20	57
Free sterols	24	22	54
7-Oxosterols	24	27	49
7α-Hydroxysterols	25	27	48
7β-Hydroxysterols	25	22	58

Similar steroids oxidized at C-7 were isolated from another Chinese drug, *Bombyx cum Botryte*, which is also used for the treatment of various cancerous diseases, [5]. Cheng *et al.* [6] tested the cytotoxicity and antitumour properties of several 7-oxo- and 7-hydroxysterols isolated from this drug or synthesized from commercial sterols on hepatoma cell cultures. According to their results, the most active antitumour compound of those isolated from *E. fischeriana* is 7β-hydroxycampesterol. 7β-Hydroxystigmasterol and 7α-hydroxystigmasterol show little antitumour activity, whereas all other constituents isolated from this plant show very little or no cytotoxicity at all [6].

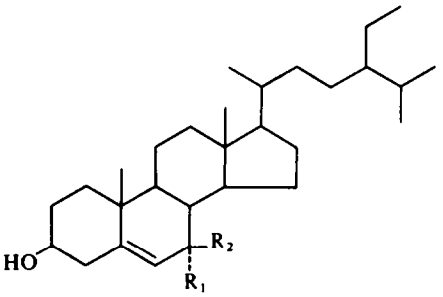
The similar therapeutic uses of these two Chinese drugs, *Bombyx cum Botryte* and *E. fischeriana*, might not be fortuitous as both contain similar antitumour steroids. Nevertheless, it is not certain that *E. fischeriana* is really a very active antitumour drug as it contains only small amounts of relative weak active compounds. However, its activity might be greater than the small amounts of isolated antitumour compounds suggest, because such drugs are often employed as mixtures and synergic phenomena between several constituents of a drug or a



1a
1b
1c
1d



2a
2b
2c
2d



3a $R_1 = R_2 = H$
3b $R_1 = R_2 = O$
3c $R_1 = OH$; $R_2 = H$
3d $R_1 = H$; $R_2 = OH$

mixture of drugs might be involved. Furthermore, a crude drug or a total extract containing only small amounts of active compounds might show better bioavailability than the pure steroids tested on HTC cultures.

EXPERIMENTAL

Most of the techniques used in the present work were previously described [10, 11]. ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS ($\delta = 0$) as int. standard on a 250 MHz spectrometer. Alcohols were silylated with BSTFA–pyridine (1:1) at room temp. for 10 min. The HTC cytotoxicity test was described in a previous paper [12].

Dry roots of *E. fischeriana* were obtained from the drug market of Hong Kong, and extracted by Hoffmann-La-Roche (Basle) successively with petrol, Et_2O and MeOH. The composition of the petrol and Et_2O extracts was similar. The MeOH extract also contained the same compounds in addition to more polar compounds such as D-glucose, fructose and sucrose, which were identified by GC of their TMSi-ethers. No diterpenes, alkaloids or flavonoids could be detected in any of the three extracts.

The petrol extract (1.6 g) was fractionated on a Si gel column using cyclohexane containing increasing amounts of EtOAc and MeOH. Fractions containing sterol esters (300 mg), free sterols (4.9 g) and polar steroids (1.7 g, eluted with MeOH) were obtained. The sterols from the free sterol and sterol ester fractions were purified by TLC and analysed as previously described [10, 11]. The MeOH fraction was further separated on a Si gel column using toluene containing increasing amounts of Me_2CO. Two fractions were recovered: a mixture of the 7-oxosterols (72 mg) and a mixture of 7α-hydroxysterols (108 mg). 7β-Hydroxysterols ($R_f = 0.40$) were separated from the 7α-hydroxysterols ($R_f = 0.28$) by TLC using $CHCl_3$–MeOH (95:5) as eluant.

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A PHENOLIC GLUCOSIDE FROM THE SEEDS OF *CARUM COPTICUM*

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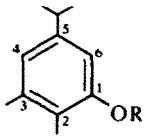
Key Word Index—*Carum copticum*; Umbelliferae; phenolic glucoside; 2-methyl-3-glucosyoxy-5-isopropylphenol; structure elucidation.

A continuation of our study of Indian medicinal plants [1, 2] has led to a chemical investigation of the seeds of *Carum copticum* and the isolation of a new phenolic glucoside, 2-methyl-3-O-β-D-glucosyloxy-5-isopropylphenol (**1**). *C. coptium* is cultivated both in the Mediterranean region and India and is known for its medicinal properties [3].

1, mp 177–8°, M$^+$ 328, was obtained as a colourless powder. The spectral studies of **1** and its acetate, **2** showed the presence of two protons, a hydroxyl, a glucosyl, a methyl and an isopropyl group substituted in a benzene ring. A positive Gibb's test of **1** and of its acid hydrolysed methyl ether, **4** indicated that the glucosyloxy group is *meta* to the phenolic hydroxyl. If the glucosyloxy group was *ortho* to the hydroxyl, the two protons would appear as doublets of J = 10 Hz each. The appearance of two singlets at δ 6.82 (1 H) and δ 7.0 (1 H) confirms the presence of a glucosyloxy group at the 3-position in the phenol nucleus leaving positions 2 and 5 for the methyl and isopropyl groups. The possibility of an isopropyl group at position 2 was ruled out by direct comparison of the derivative **6** (see Experimental) with an authentic sample of thymol (2-isopropyl-5-methylphenol) methyl ether. Permethylation [5] and hydrolysis of **1** gave 2,3,4,6-tetra-*O*-methyl-D-glucopyranose establishing that C_1 of the glucose is linked with the aglucone, **3**. Finally, the β-linkage of the glucose was confirmed by enzymatic hydrolysis.



1 R = H, R_1 = OGlc.
2 R = Ac, R_1 = −OGlc·Ac_4
3 R = H, R_1 = −OH
4 R = Me, R_1 = OH
5 R = Me, R_1 = −O·SO_2·C_6H_4·Me−*p*
6 R = Me, R_1 = H

EXPERIMENTAL

UV spectra were recorded in MeOH and in the NMR spectra TMS was used as internal standard.

Extraction and Isolation. Dried *C. copticum* seeds (3.0 kg) were extracted successively with petrol, C_6H_6 and EtOH. The C_6H_6 insoluble portion of the EtOH extract was concd and chromatographed on a Si gel column (400 g) with a $CHCl_3$ → MeOH gradient. The fractions eluted with $CHCl_3$–MeOH (23:2) on prep. TLC (Si gel, EtOAc–MeOH–H_2O, 100:16.5:13.5) yielded the glucoside, **1** (500 mg).

Identification of **1** R_f s on Si gel: 0.43 (EtOAc–MeOH–H_2O, 100:16.5:13.5); 0.55 ($CHCl_3$–MeOH, 7:3); (Found: C, 58.2; H, 7.7.